Signal and Noise: Garbage in Garbage out

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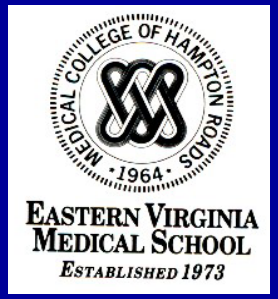
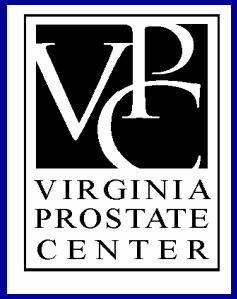
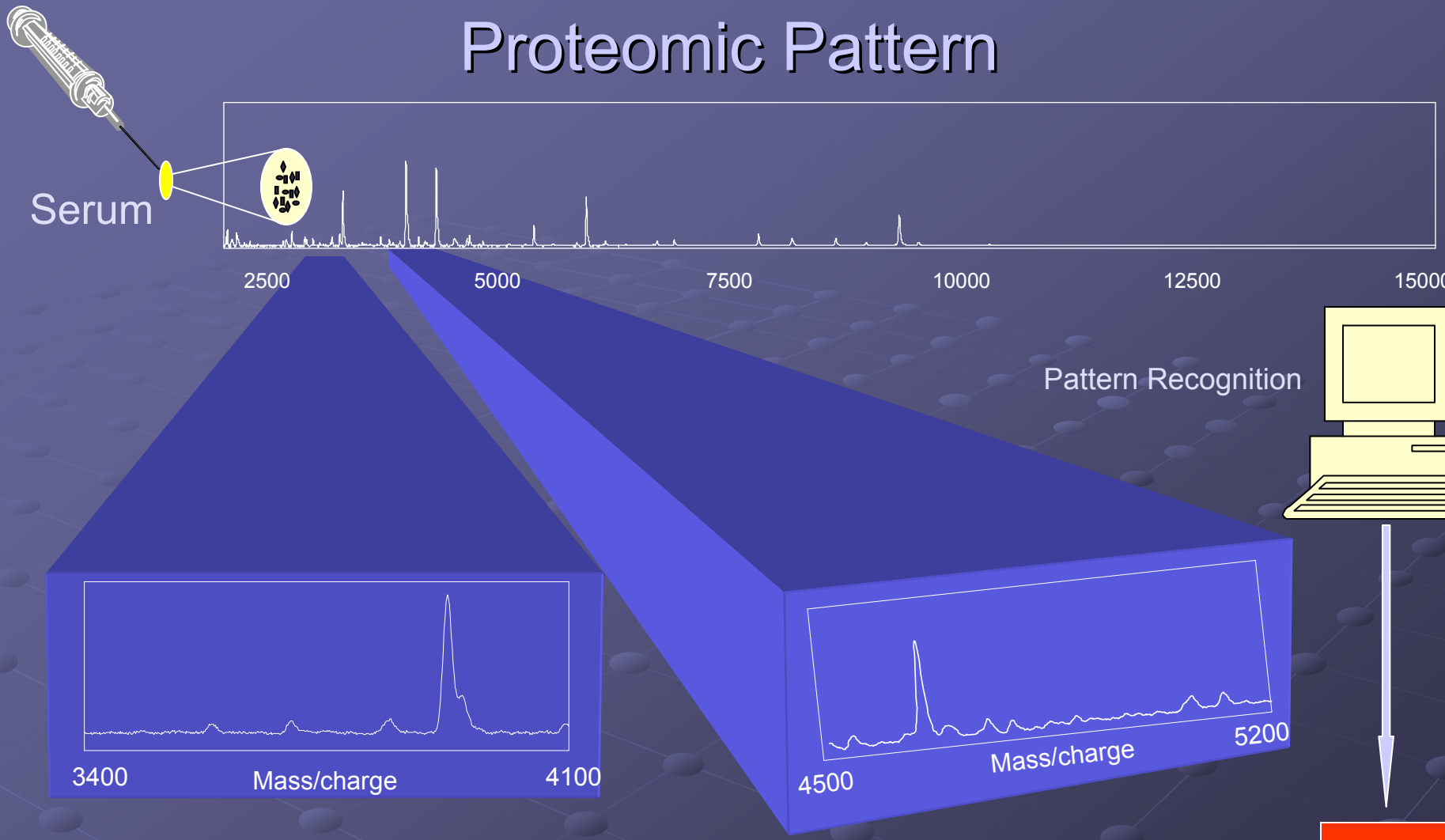




Proteomic Pattern



The discriminating pattern formed by a subset of proteins or peptides buried among the entire repertoire of thousands of proteins represented in the serum sample.

THINGS WE CAN DO TO MAKE THE PATTERNS BETTER

1. Some things to do before the Mass Spec (TOF-tube)
2. Some things to do after the Mass Spec (TOF-tube)

Achieving Reproducibility in SELDI

1. Get a gatekeeper for experimental design and interpretation.
2. Use “standard” samples; external (spiked) proteins, internal proteins, serum sample for QC.
3. Synchronization/optimization of instrument output using the QC sera. Laser/detector settings.
4. Constant monitoring and adjustment of parameters.
5. Automation of sample processing steps.
6. Find out what the peaks are! (robustness)

Table 1. QC spectra criteria

Protein	Signal to Noise Ratio (S/N)	Resolution
Insulin	N/A	600
IgG	700	N/A
Peak1: 5906 ± 0.2%	>40	>400
Peak 2: 7768 ± 0.2%	>80	>400
Peak 3: 9289 ± 0.2%	>80	>400

Quality control assessment of the PBSII is based on signal to noise ratio (s/n) and resolution. The table provides the s/n and resolution required for each QC protein used in Phase IA for a site to proceed to Phase II.

QC standardization
At six Validation Sites

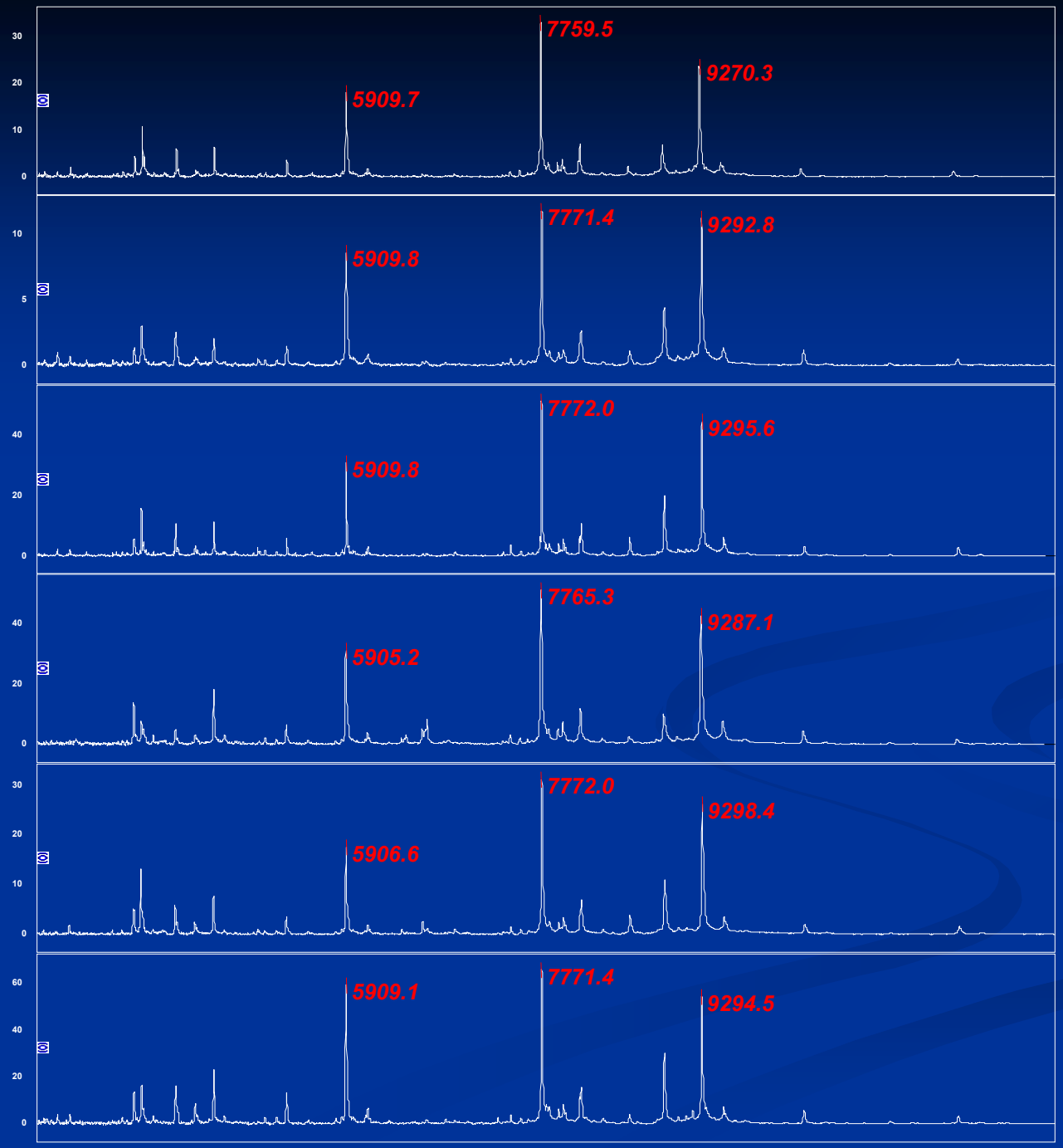
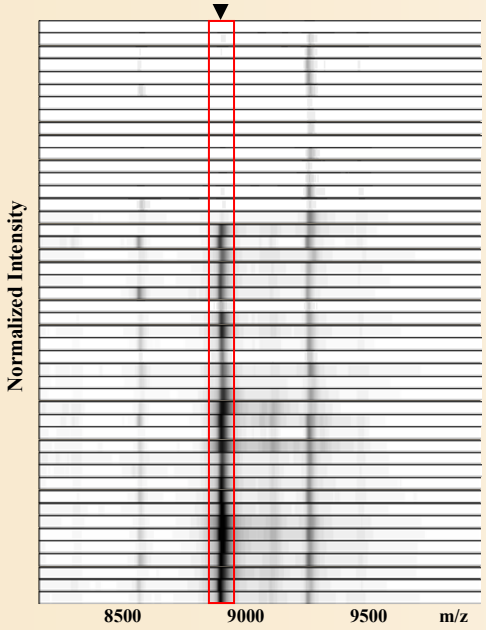


Table 2b Inter-Lab variability

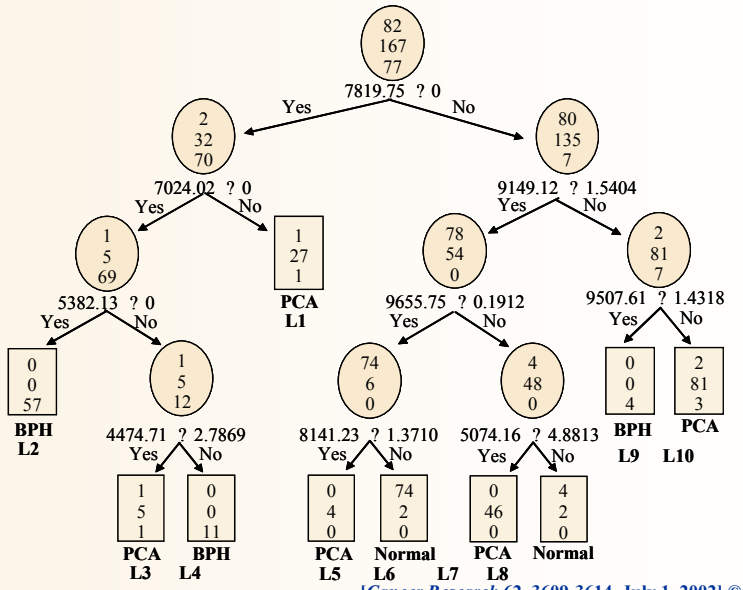
		Mass	Intensity	S/N	Resolution
Peak 1	**average**	5906.47	26.57	163.06	460.73
	stdev	6.70	9.67		107.72
	CV	**0.0011**	**0.36**		**0.23**
Peak 2	**average**	7768.61	35.94	242.75	505.54
	stdev	8.41	6.25		82.77
	CV	**0.0010**	**0.17**		**0.16**
Peak 3	**average**	9289.18	30.96	244.03	439.28
	stdev	9.89	4.70		77.35
	CV	**0.0011**	**0.15**		**0.18**

Summary of Biomarker Discovery and Identification

SELDI-TOF



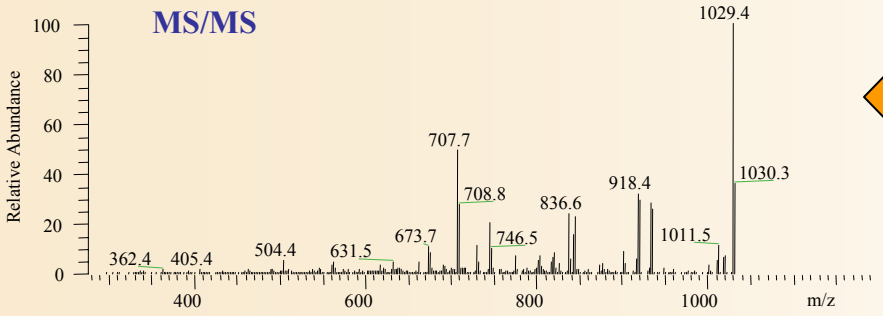
Classification and Regression Tree Analysis



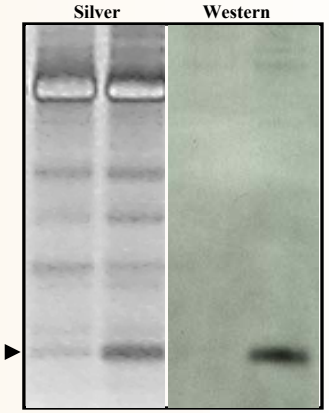
[*Cancer Research* 62, 3609-3614, July 1, 2002] © 2002 American Association for Cancer Research

Identification

MS/MS



Purification



CENTER FOR BIOMEDICAL PROTEOMICS
EASTERN VIRGINIA MEDICAL SCHOOL

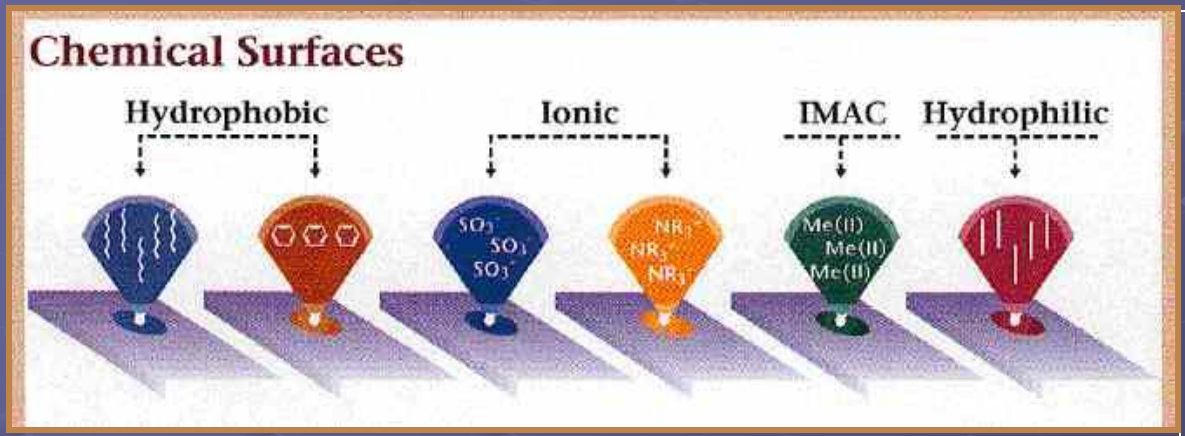
Things to do after the tube

Analysis of Source of Variation

1. Metrologic Analysis of SELDI-TOF Process.
2. Spectral Analysis of Output.

Proteomics Using SELDI Technology

Surface Enhanced Laser Desorption



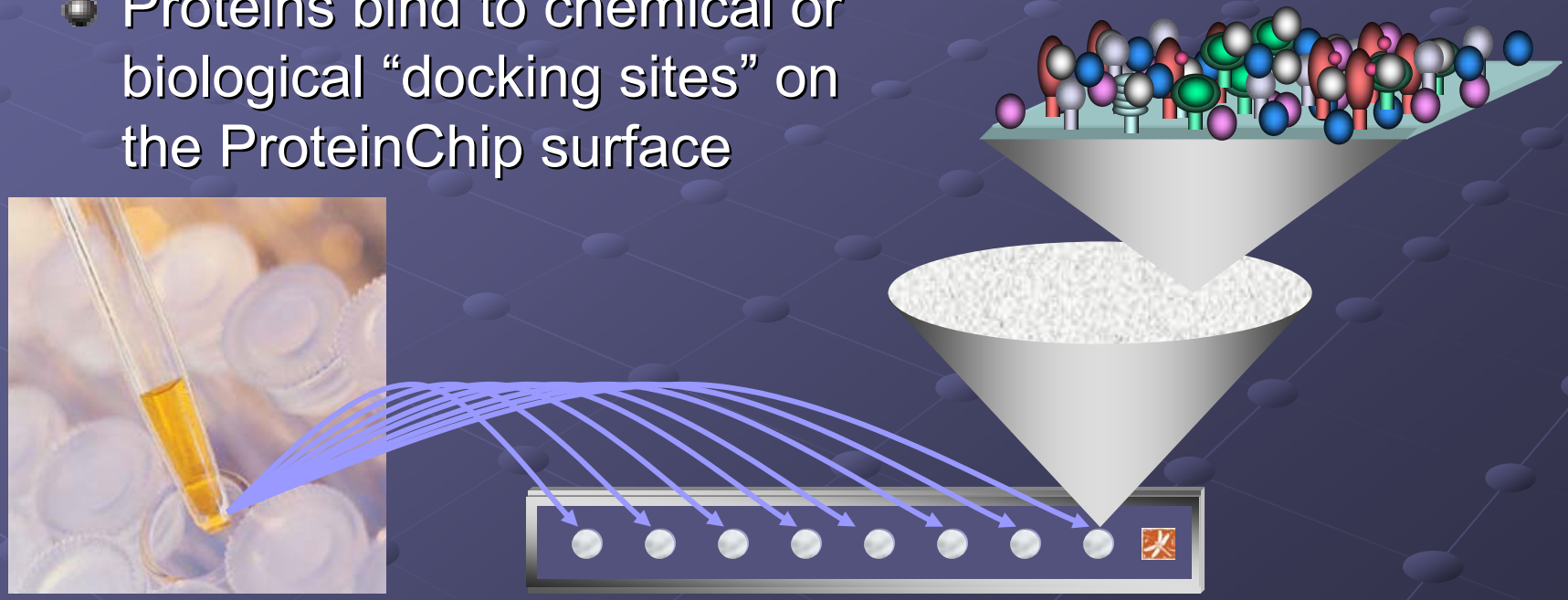
←Surface Chemistries Each chip binds a specific set of proteins based on the chromatographic surface of the ProteinChip®.

←Protein Chips

Each spot on the chip will contain sample from a control or diseased/treated source. The spots are analyzed separately and a mass spectra is created for each spot representing the proteins bound to the chip surface.

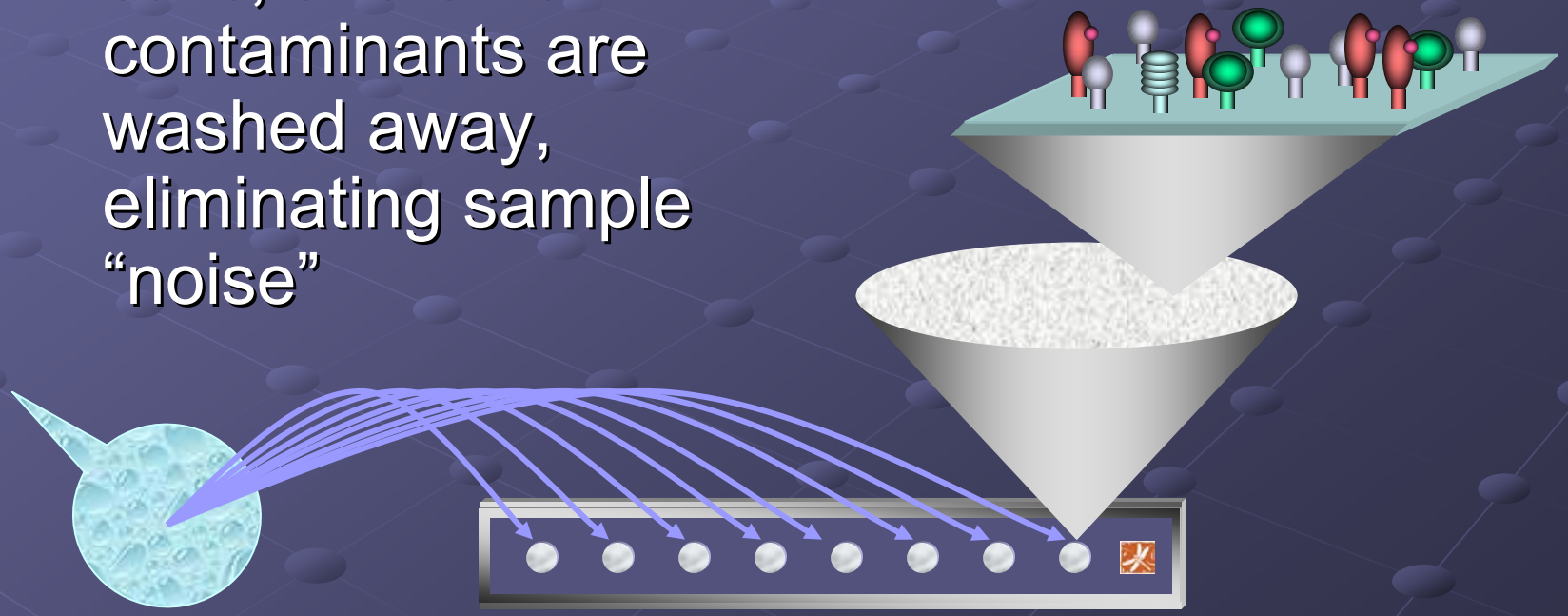
ProteinChip Technology: **Protein Binding**

- Crude sample is placed (and processed) on a ProteinChip Array
- Proteins bind to chemical or biological “docking sites” on the ProteinChip surface



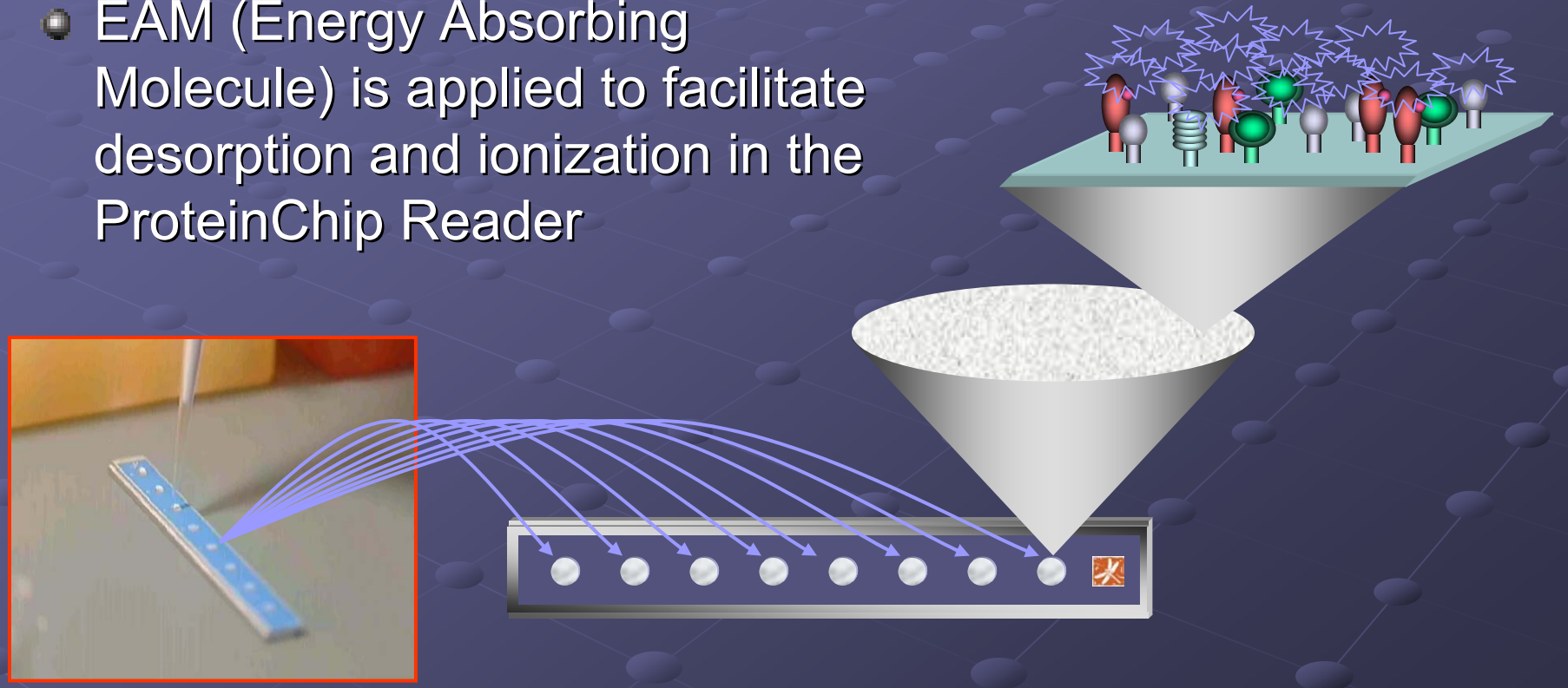
ProteinChip Technology: **Washing Reduces Non-Specific Binding**

- Non-binding proteins, salts, and other contaminants are washed away, eliminating sample "noise"

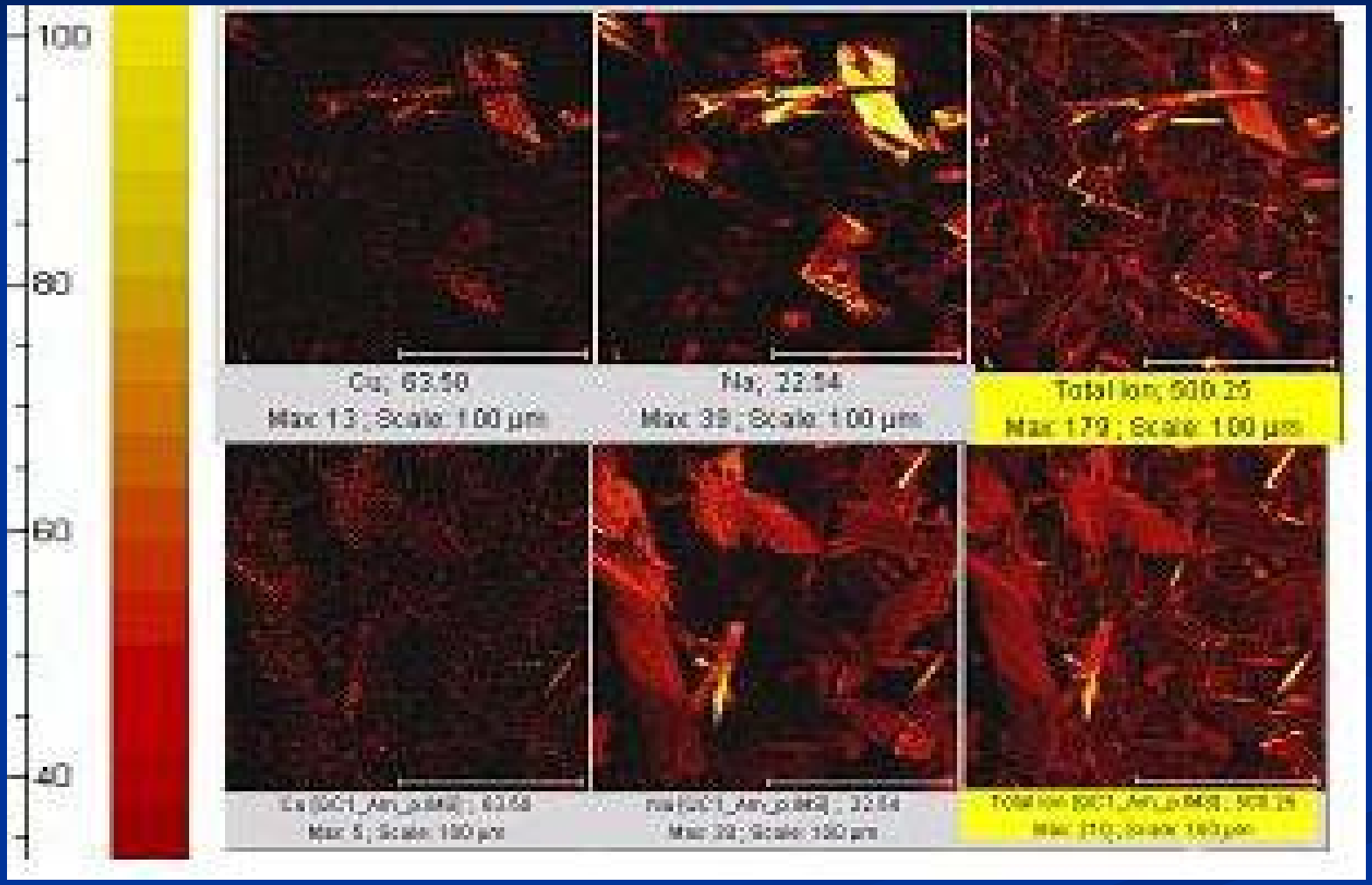


ProteinChip Technology: **Addition of EAM**

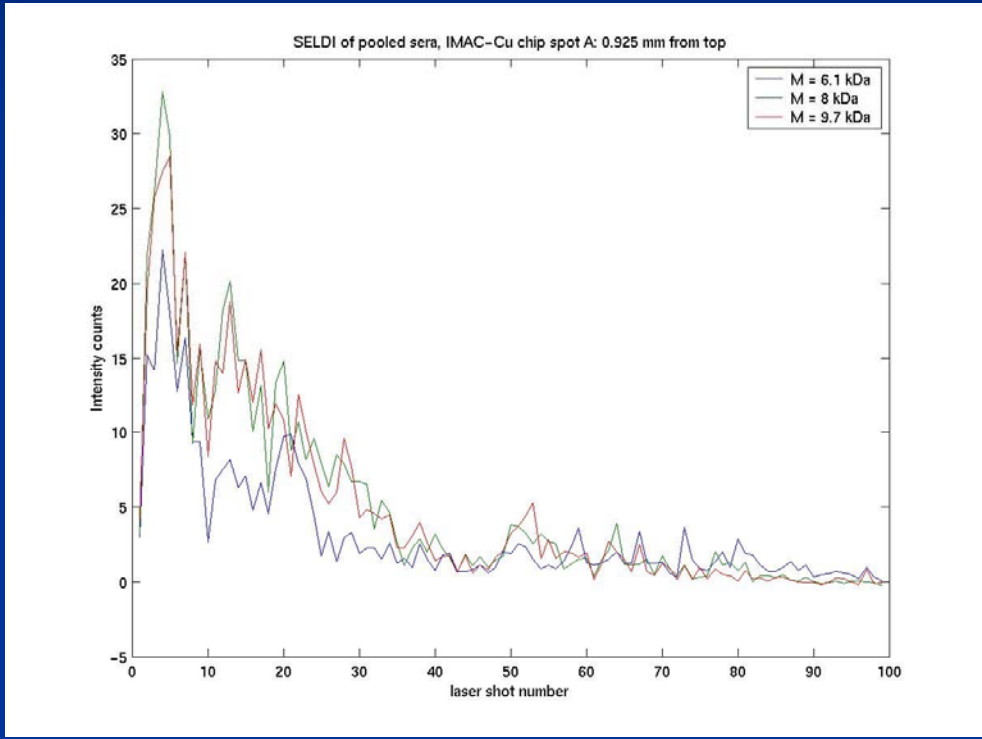
- EAM (Energy Absorbing Molecule) is applied to facilitate desorption and ionization in the ProteinChip Reader



Desorption Surface

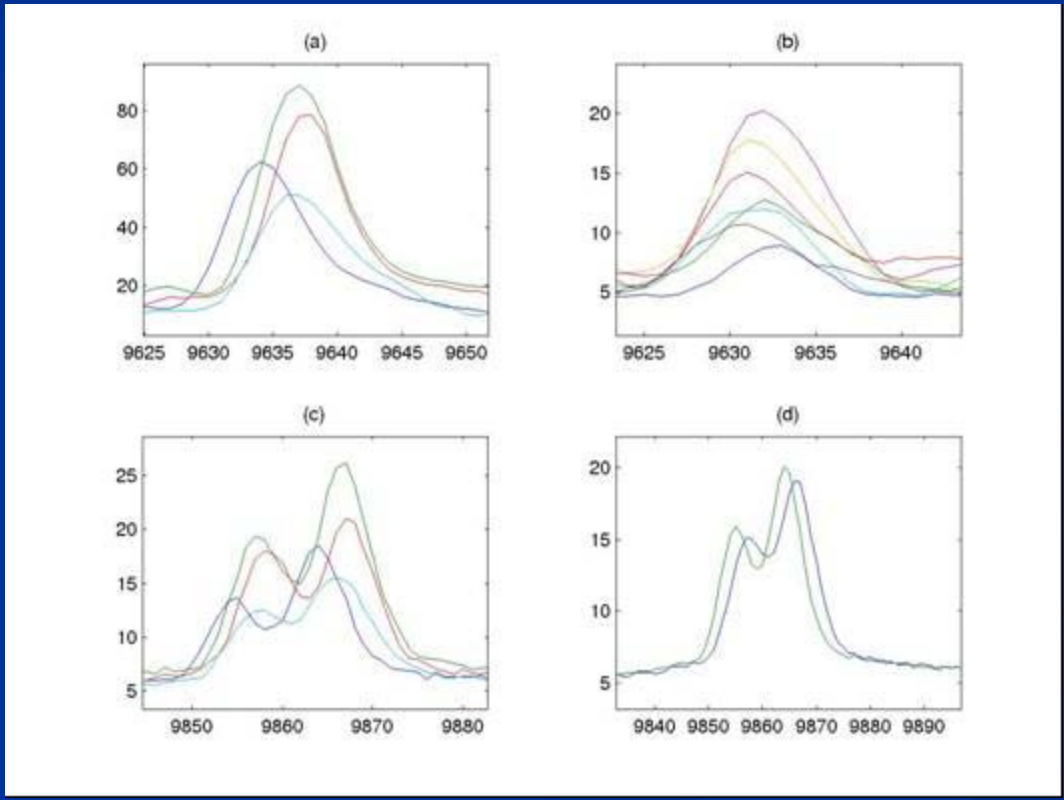


Limited Inefficient Desorption

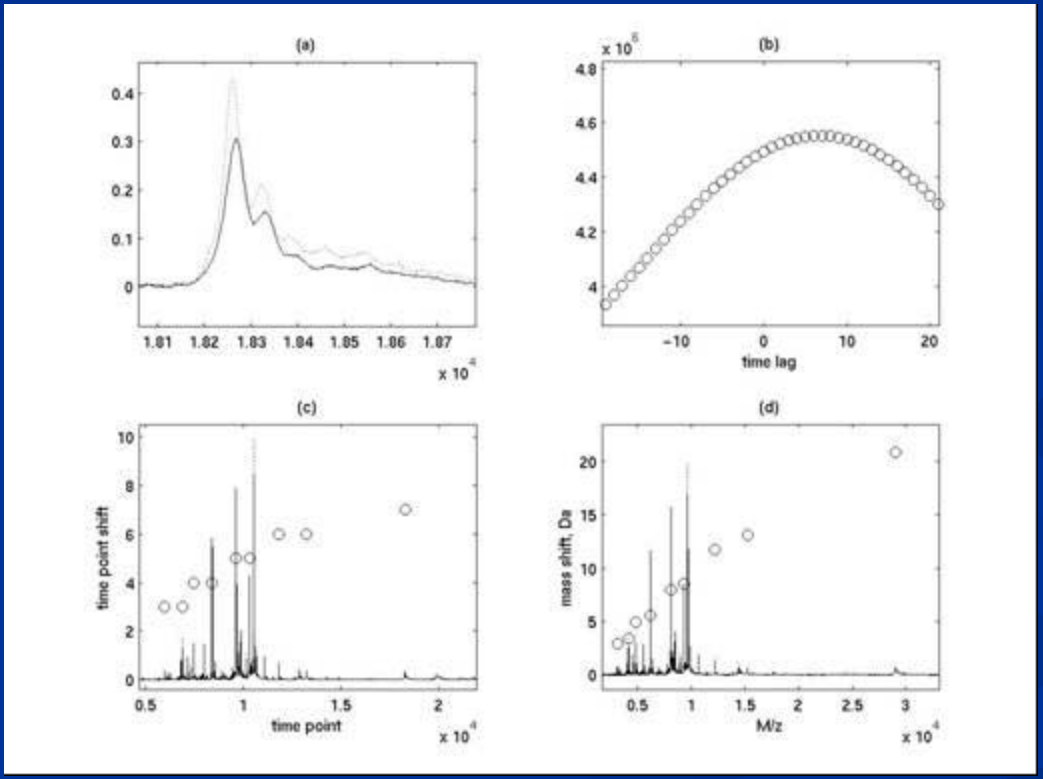


Nano-Scale Surface Polishing

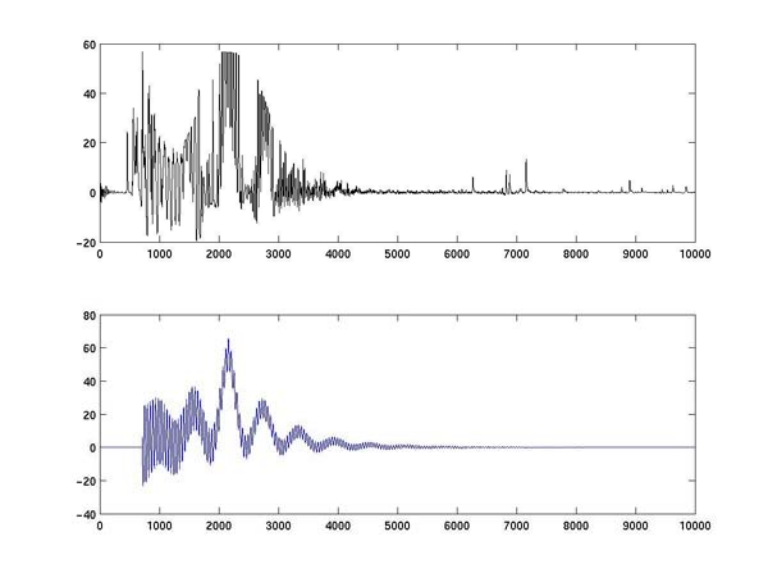
Peak Jitter Between Single Laser Shots Reduced Resolution

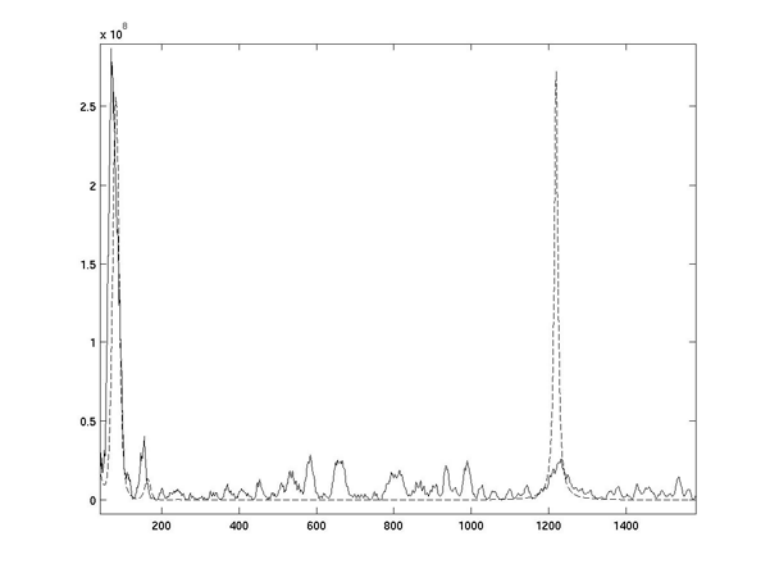


Automatic Dejitter

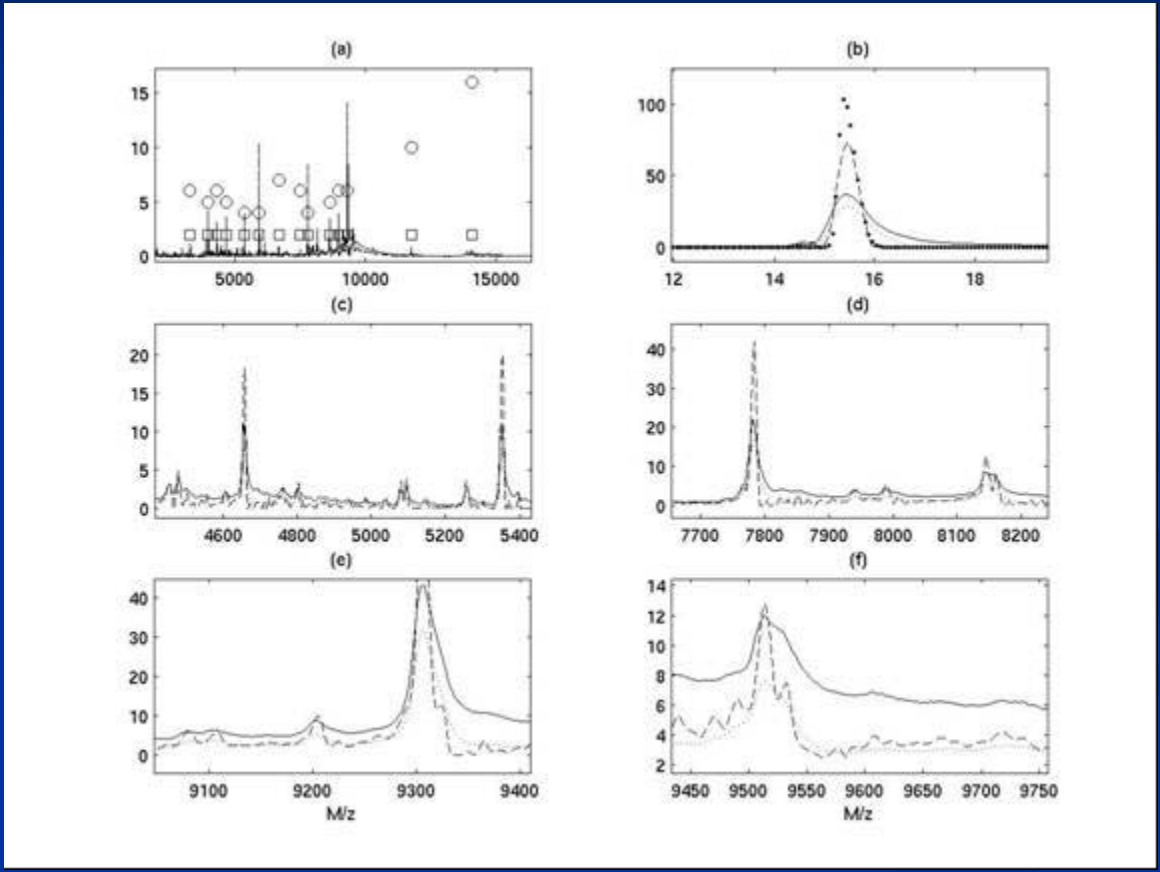


Denoising Filters



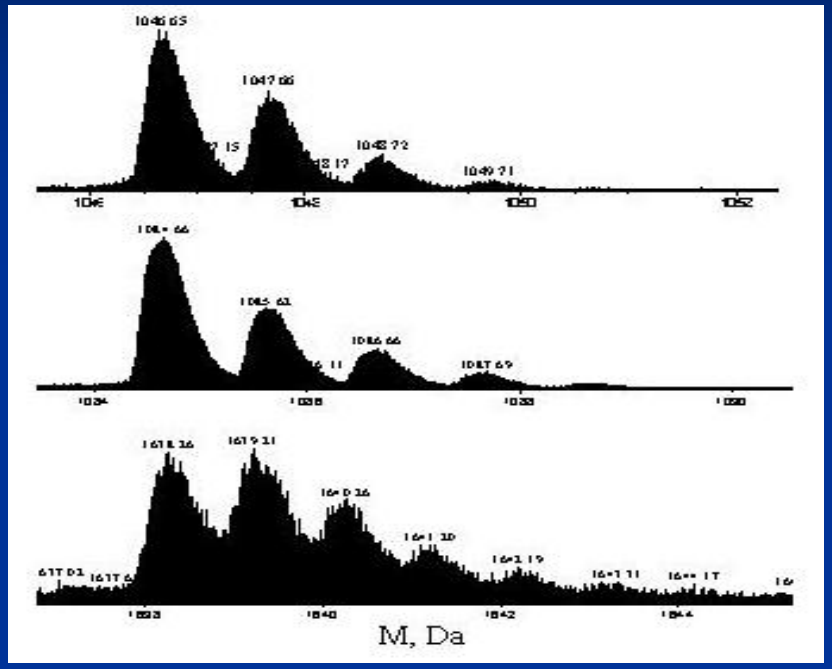


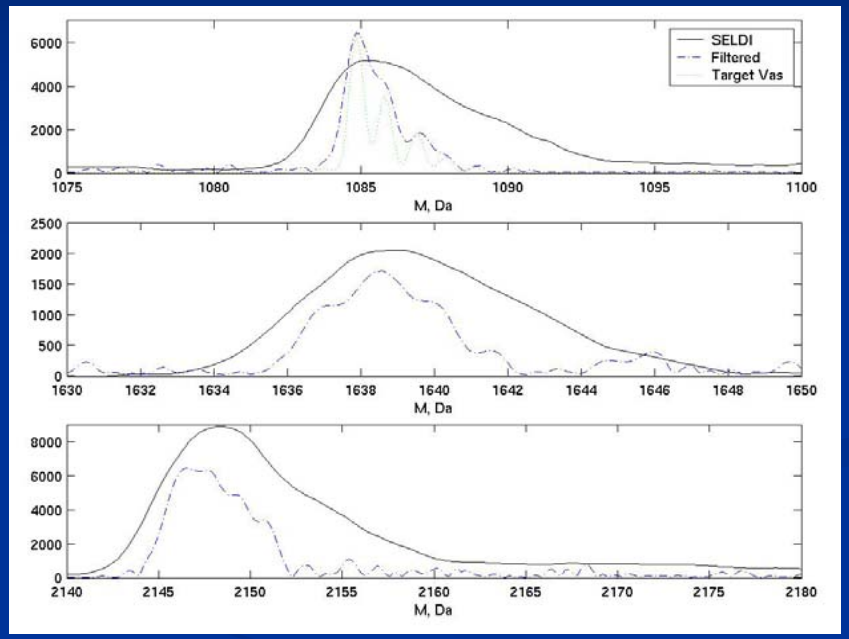
Trace Add-Back Filters



Model based target filtering

Trace Add-Back Filters





Peptide standards with SIMS-resolved isotopic structure

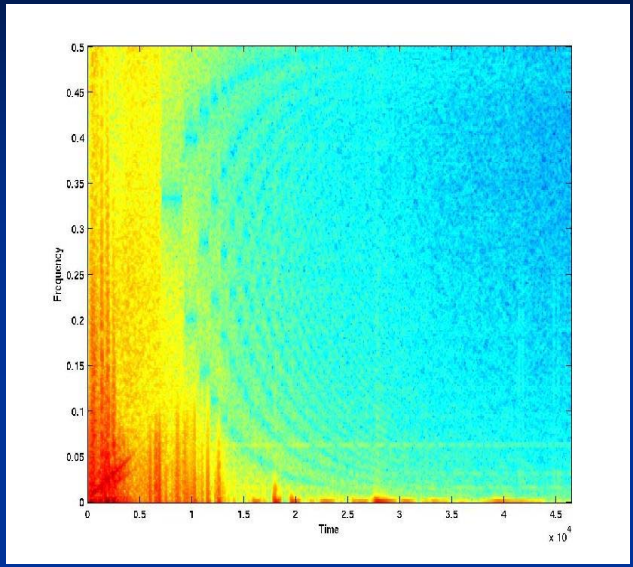
Best approach may involve

Internal Standards with known isotope structure

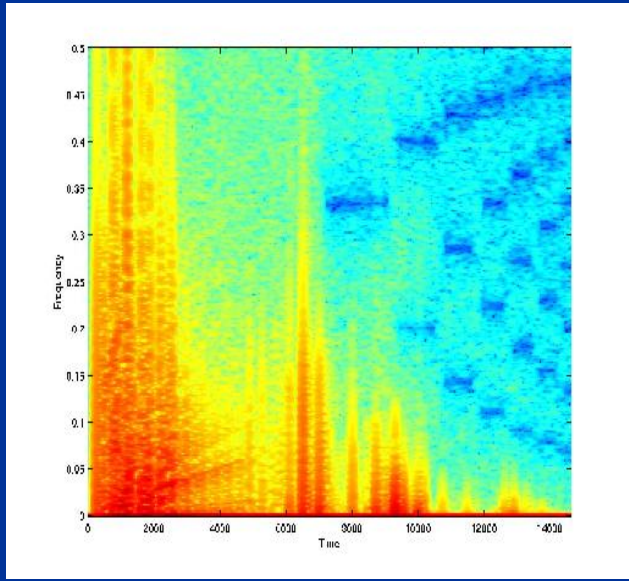
Placing external proteins in data valleys

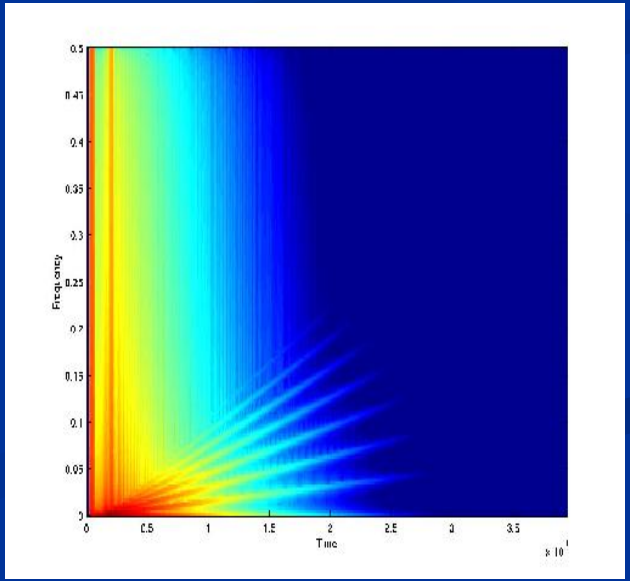
The success of denoising filters depends on defining baseline

Spectral Analysis

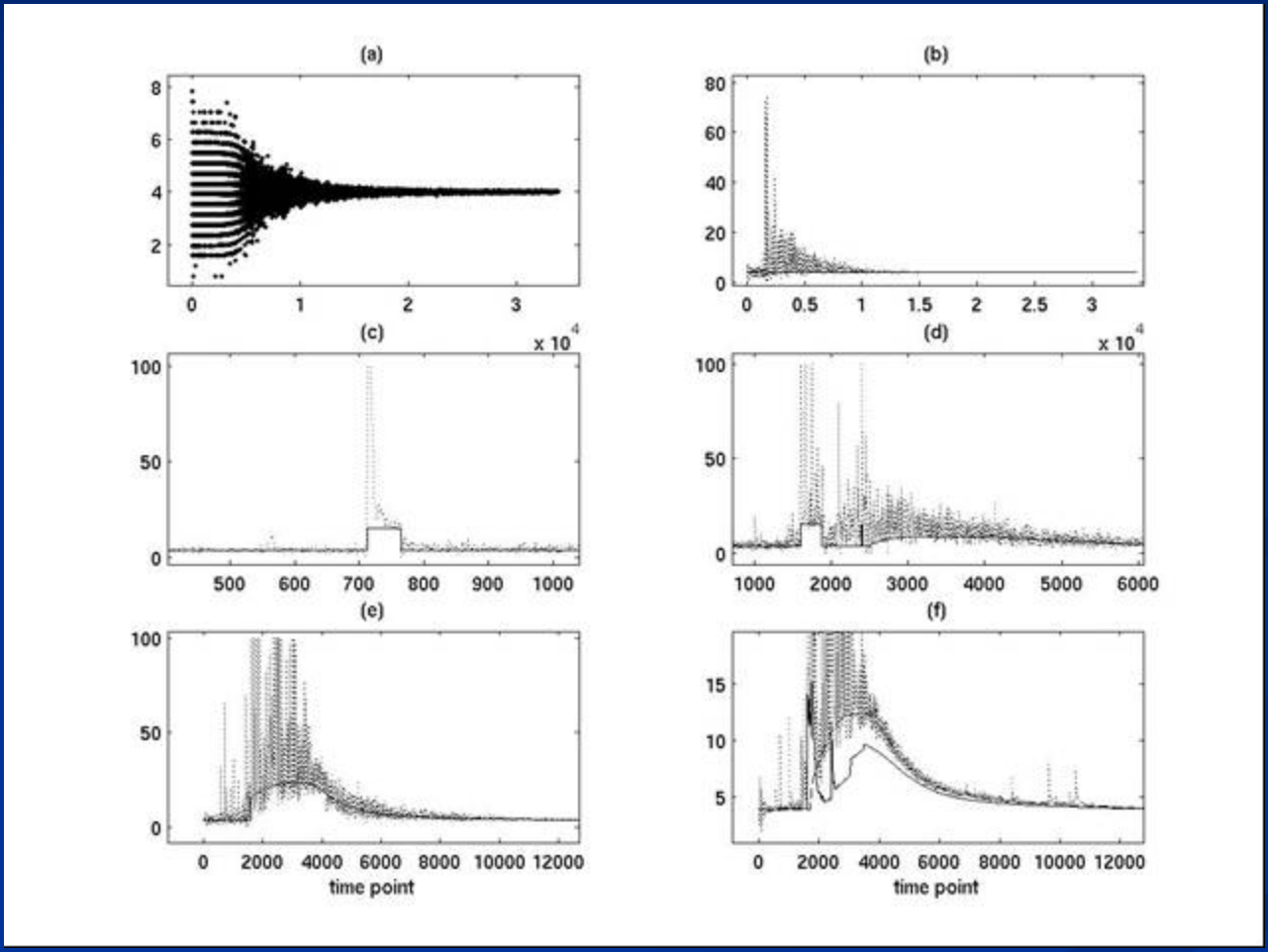


Detector Overload



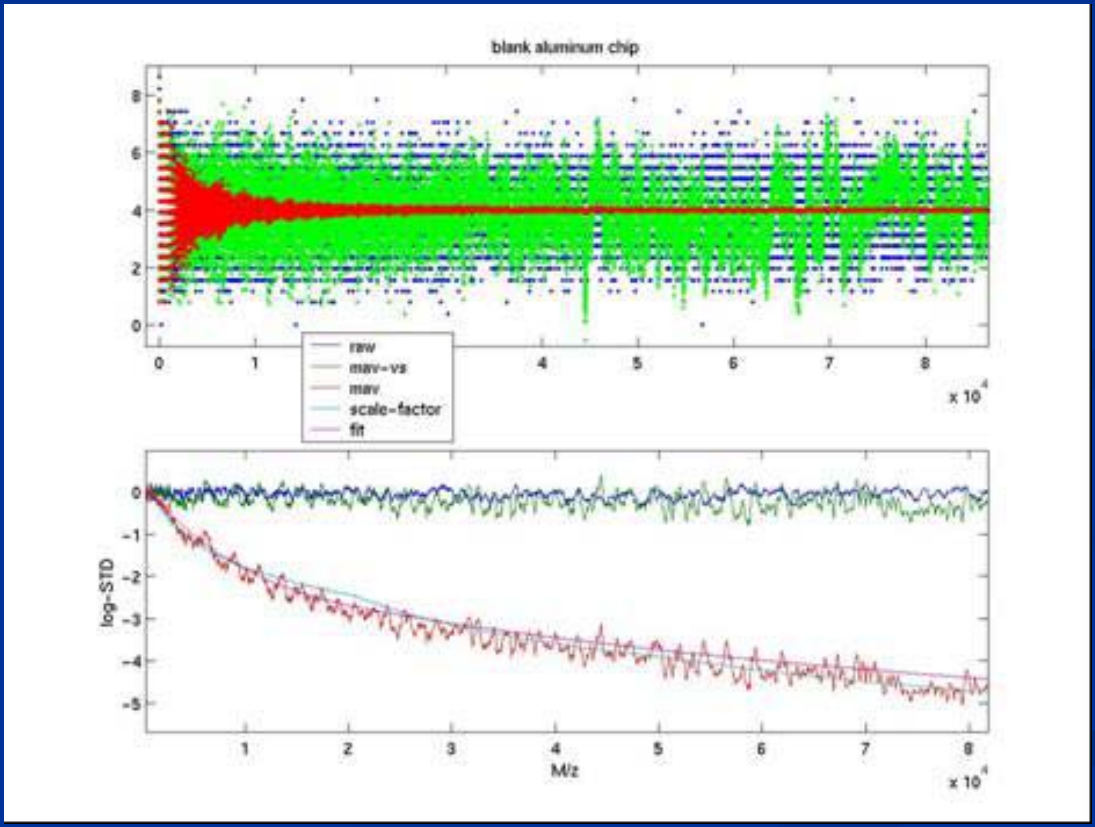


Effect of Detector Overload On Baseline

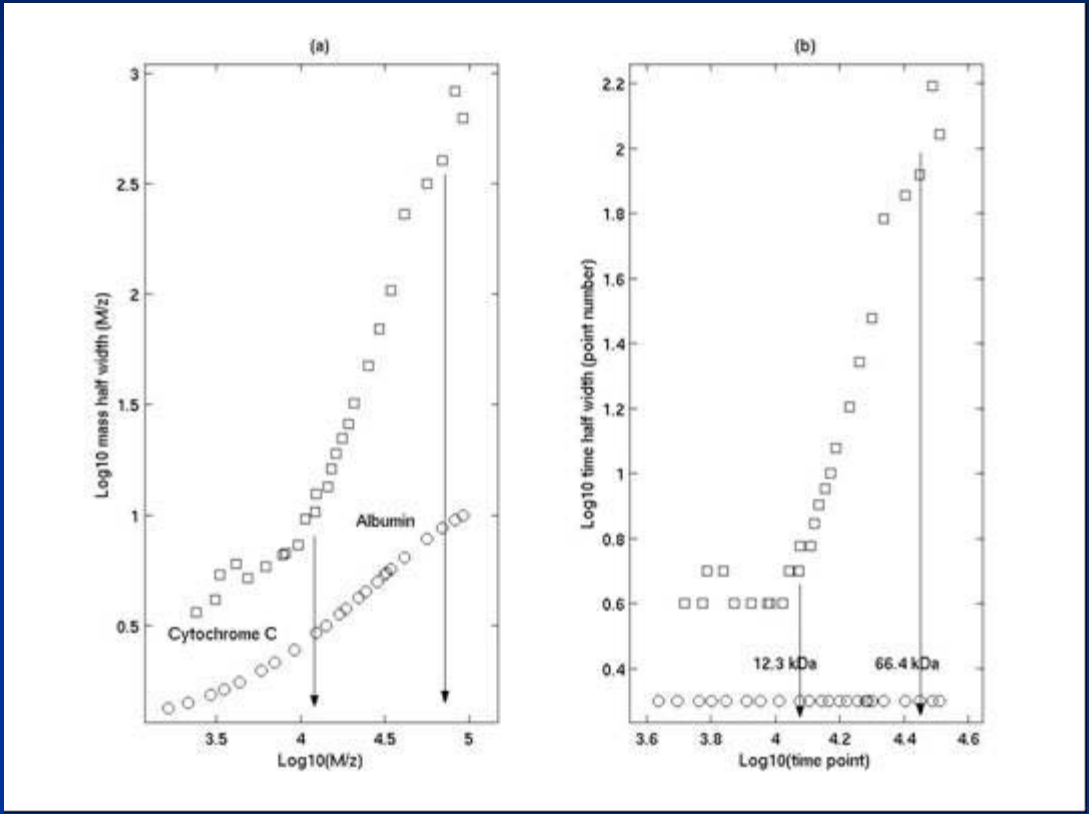


Variance Rescaling

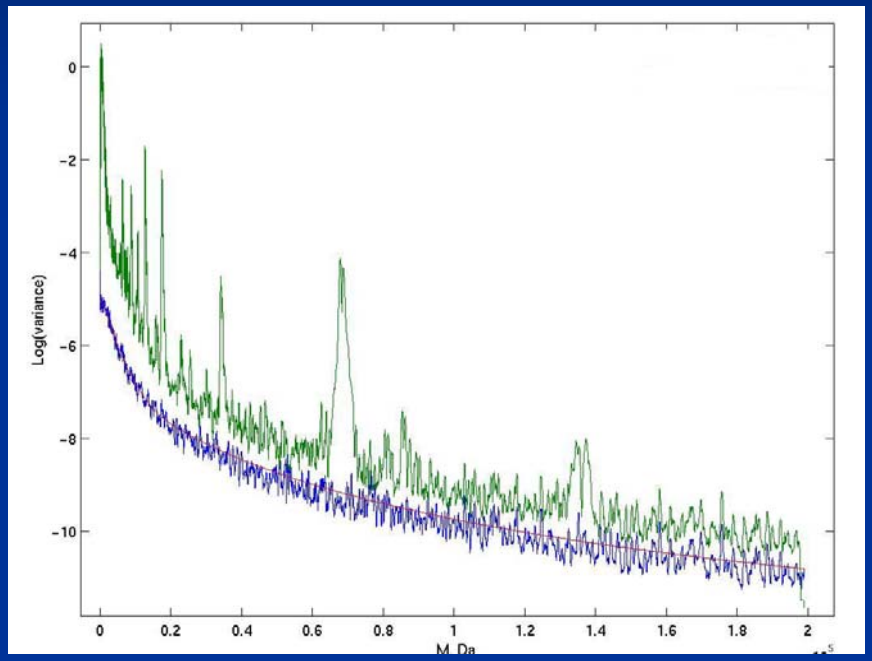
Effect of Default Moving Average Filter

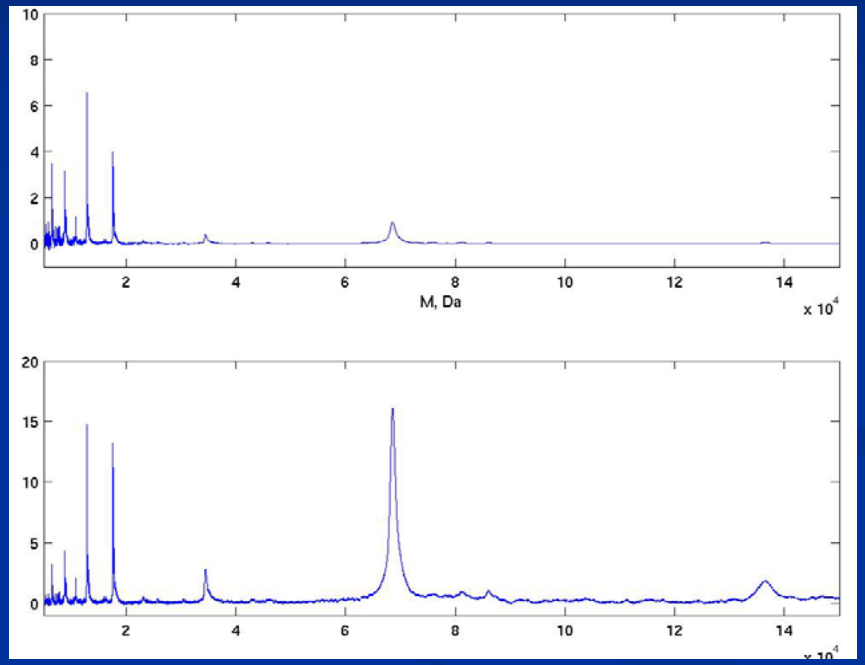


Mass dependence of peak width and default MAV

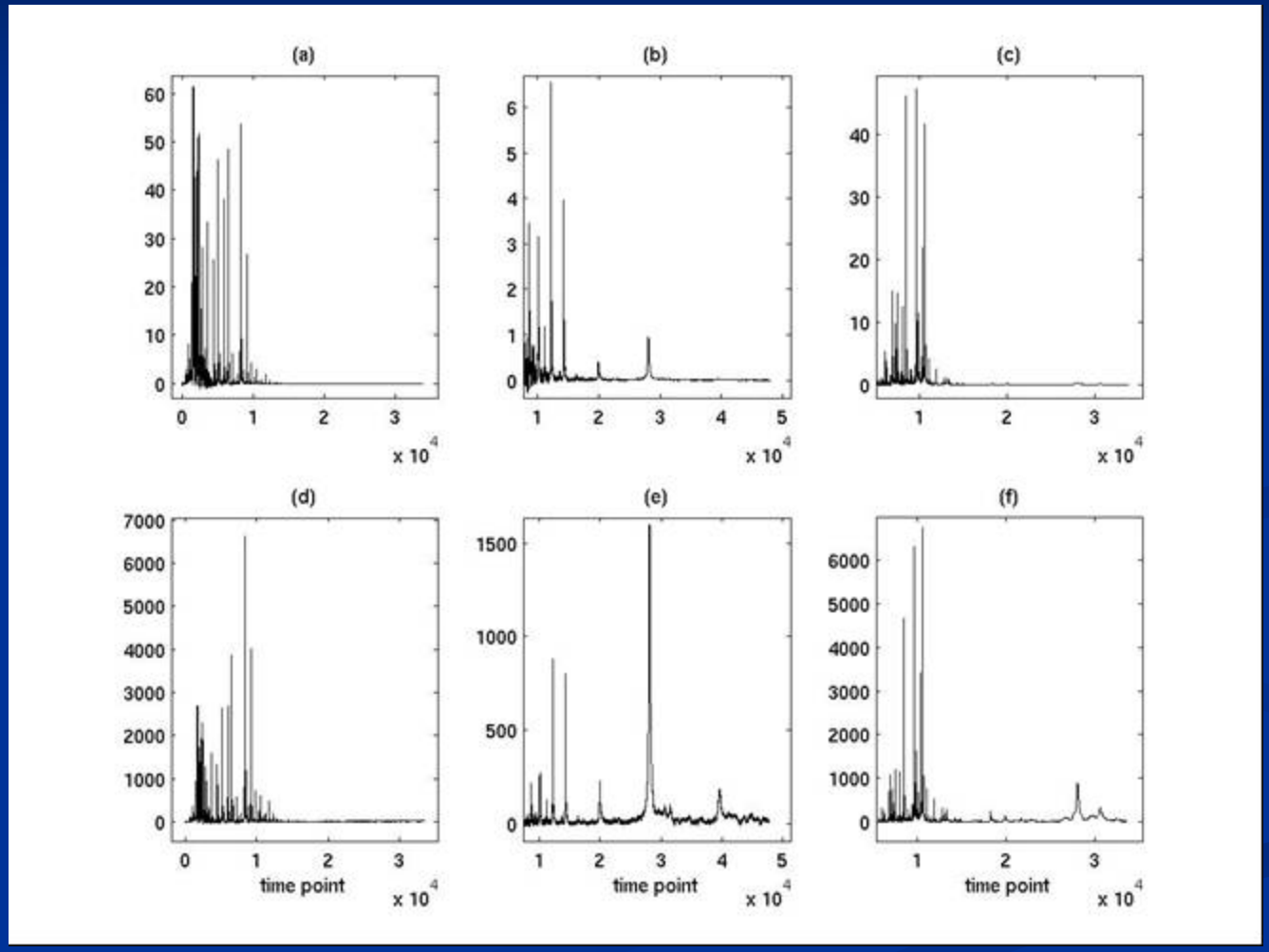


Mass Dependence of Variance



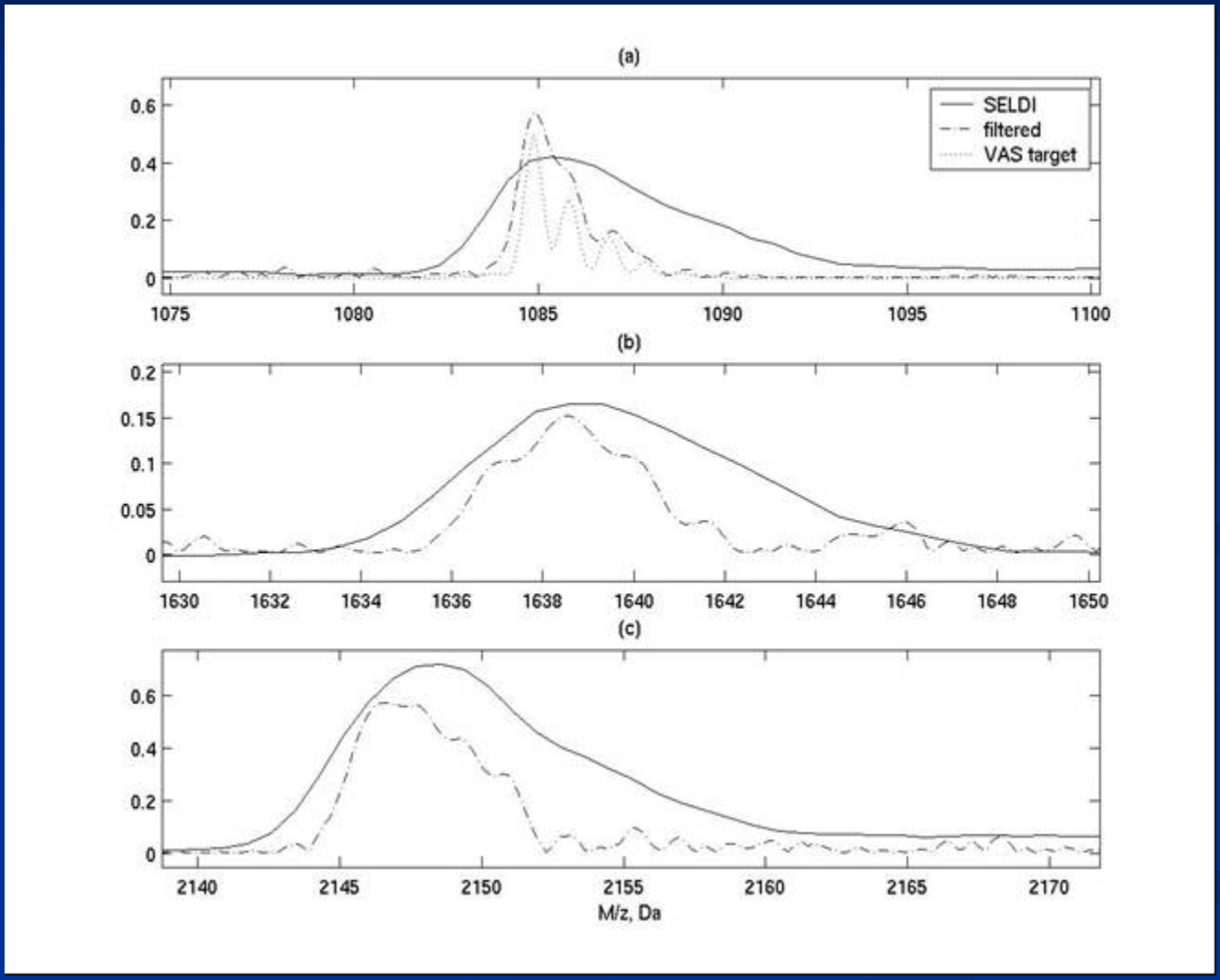


Variance Rescaling: Stationary Noise, Increased Sensitivity

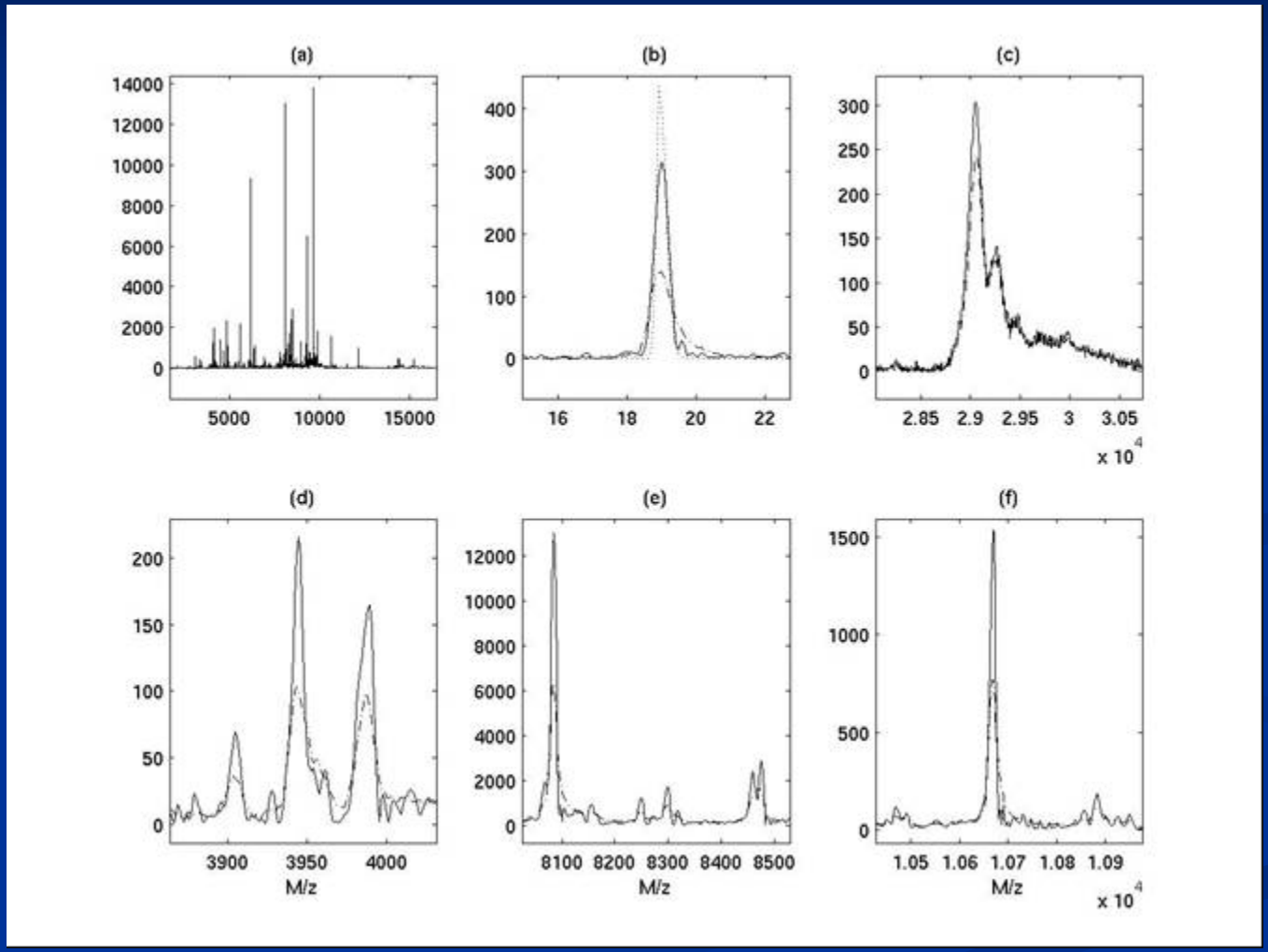


Putting it all together

Enhanced Resolution of Calibrant Peaks

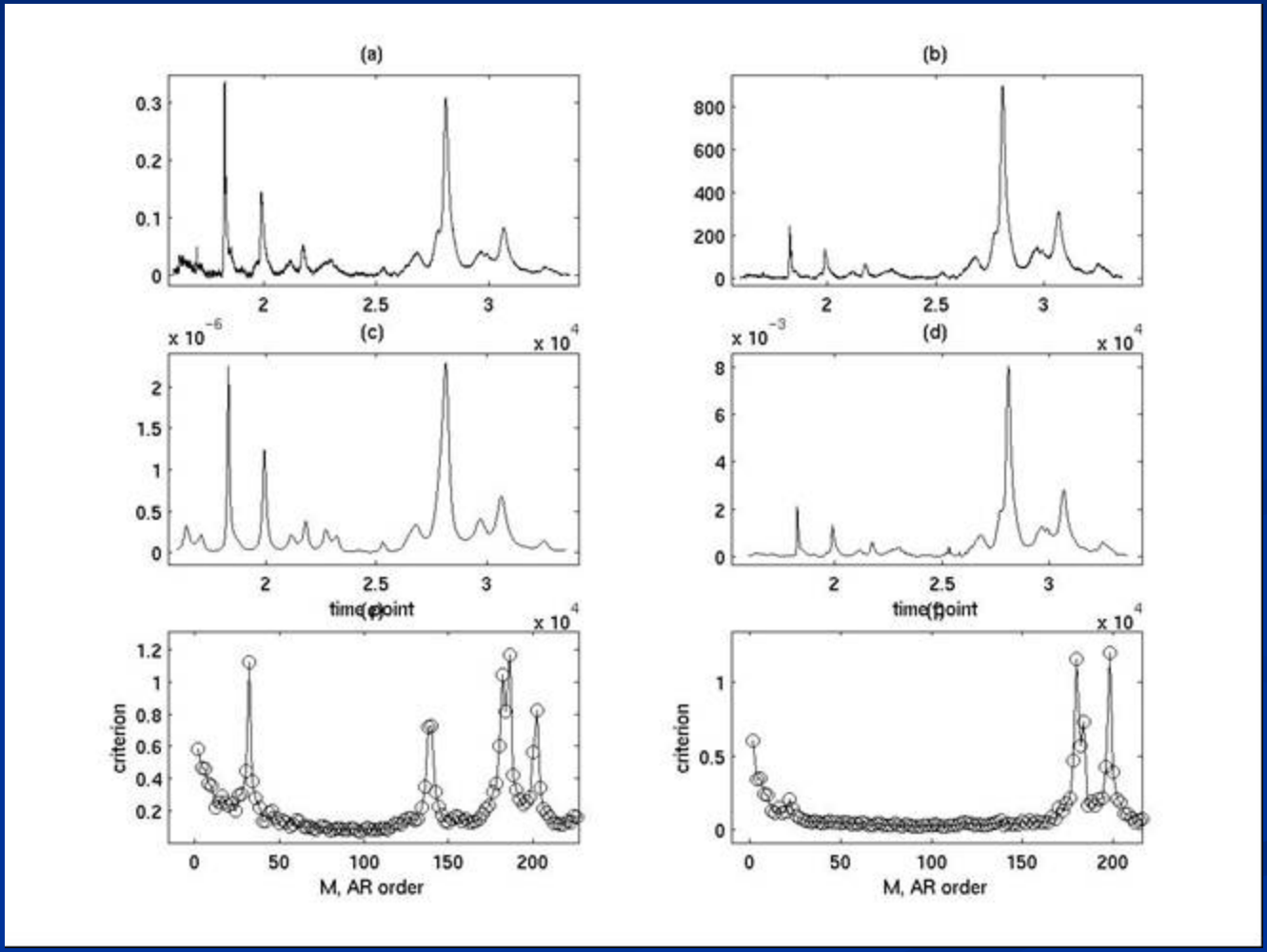


Enhanced Resolution in Pooled Serum



Default

BKG-Sub, MAV, Var-Rescale



Summary

Improving the processing of data output can dramatically improve sensitivity, resolution and reproducibility.

The Fold improvement may equal that of the “High resolution” SELDI-QStar.

Lookout for default Settings

Eastern Virginia Medical School
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